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Abstract

The compositions of paradoxin and taipoxin (PDx and TPx, respectively) were investigated using size exclusion chromatography (SEC) and nano-electrospray ionization mass spectrometry (nano-ESI-MS). The elution profiles from size exclusion chromatography of the venoms from *Oxyuranus microlepidotus* and *Oxyuranus scutellatus* were similar. Fractions corresponding to the trimeric toxins were treated with guanidinium hydrochloride and the individual subunits were separated by HPLC. In this report we present the size exclusion chromatography profiles for these toxins, and the nano-ESI mass spectra of the subunits after separation by HPLC: the first such comparative study of these toxins at the protein level. Data in this article are associated with the research article published in *Toxicon*: "Insight into the subunit arrangement and diversity of paradoxin and taipoxin" (J.A. Harrison, J.A. Aquilina, 2016).

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Data Article

Mass spectrometry data and size exclusion chromatography profiles of Australian taipan venom toxins



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ABSTRACT

The compositions of paradoxin and taipoxin (PDx and TPx, respectively) were investigated using size exclusion chromatography (SEC) and nano-electrospray ionization mass spectrometry (nano-ESI-MS). The elution profiles from size exclusion chromatography of the venoms from *Oxyuranus microlepidotus* and *Oxyuranus scutellatus* were similar. Fractions corresponding to the trimeric toxins were treated with guanidinium hydrochloride and the individual subunits were separated by HPLC. In this report we present the size exclusion chromatography profiles for these toxins, and the nano-ESI mass spectra of the subunits after separation by HPLC: the first such comparative study of these toxins at the protein level. Data in this article are associated with the research article published in *Toxicon*: "Insight into the subunit arrangement and diversity of paradoxin and taipoxin" (J.A. Harrison, J.A. Aquilina, 2016) [1].

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Specifications Table

Subject area	Chemistry and Biology
More specific subject area	Structural proteomics
Type of data	Table, figure
How data was acquired	Size exclusion chromatography, HPLC and mass spectrometry
Data format	Analyzed
Experimental factors	Amino acid sequences of toxin subunits were sourced from uniprot.org
Experimental features	Toxin oligomers and subunits were separated by chromatographic techniques for analysis by mass spectrometry
Data source location	University of Wollongong, NSW, Australia
Data accessibility	Data are within this article

Value of the data

- Outlines the purification steps required to separate oligomeric PLA₂.
- Presents size exclusion chromatography elution profiles for comparison with oligomeric toxins from other snake species.
- Describes isolation of individual toxin subunits for further characterization such as for sequence and post-translational modification analysis.
- Describes the instrumentation and conditions required for acquiring ESI mass spectra of the oligomeric toxins and subunits.
- Presents nano-ESI mass spectra of oligomeric toxins and subunits from *Oxyuranus microlepidotus* and *Oxyuranus scutellatus*. These spectra can serve as a comparison for those from other species.

1. Data

Three figures showing the size exclusion chromatograms for whole venom analysis and the nano-ESI mass spectra of the peaks from the HPLC of PDx and TPx subunits. Three tables, two containing the experimental subunit masses determined using nano-ESI-MS after HPLC separation, and a third showing the sequence data for the previously characterized subunits of TPx and PDx.

2. Experimental design, materials and methods

2.1. Size exclusion chromatography (SEC)

For each of the chromatographic runs, lyophilized whole venom from *O. microlepidotus* or *O. scutellatus* was dissolved in 200 mM ammonium acetate (NH₄OAc) and loaded onto a Superdex 100 10/30 size exclusion column (GE Healthcare, Uppsala, Sweden). Fig. 1 shows the elution profiles. The second fractions (labeled 2, primarily containing the trimeric PLA₂, PDx or TPx) were analyzed by SDS-PAGE (Fig. 1, insets). Each gel consisted of a 4% stacking gel and 15% resolving gel. Electrophoresis was performed using a Mini Protean 3 system (Bio-Rad, California, U.S.A), which was powered by a Bio-Rad power pack 300 power supply (Bio-Rad, California, U.S.A). Protein samples were mixed with a

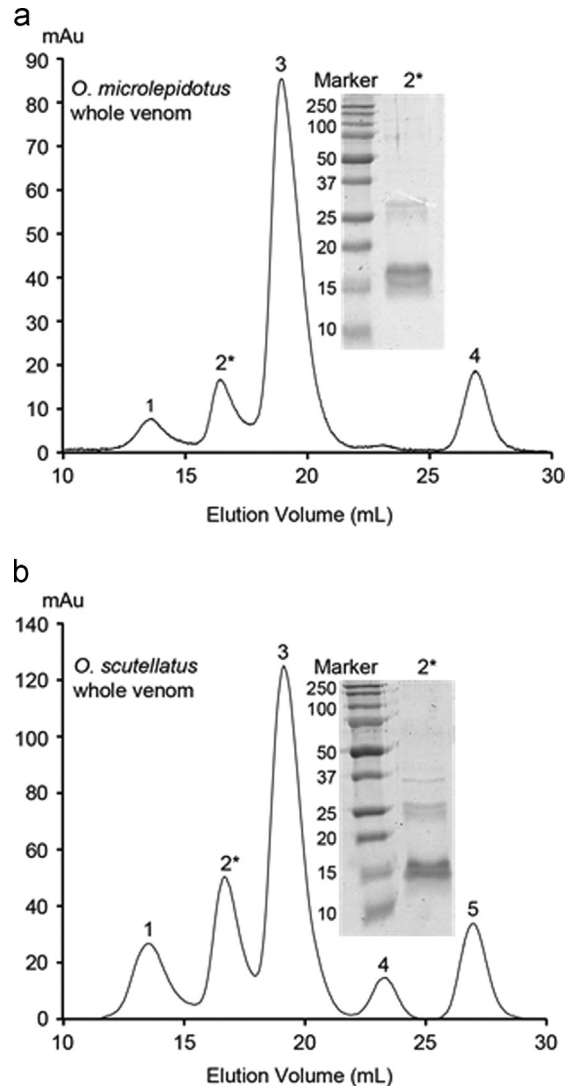


Fig. 1. Separation of ~45 kDa toxins from *Oxyuranus* spp. whole venom via SEC. Chromatograms show the constituents of *O. microlepidotus* (A) and *O. scutellatus* (B) whole venom which were partitioned by SEC. Each of the individual SEC peaks were numbered in order of elution, and those peaks previously reported to contain PDx and TPx are marked with an asterisk [2–4]. The molecular weight of each marker band is given in kDa.

cracking buffer solution (0.5 M Tris–HCl, pH 6.8: 14 mL, 0.02 g Coomassie Blue, glycerol: 16 mL, 10% SDS: 16 mL) at a ratio of 1:1, then heated to 100 °C for 5 min prior to loading 10 µL of each sample into individual wells. Molecular weight standards were 5 µL of Precision Plus Protein™ Dual Color Standards (Bio-Rad, California, U.S.A.). Separation was achieved at 120 V after 1 h. Gels were stained with

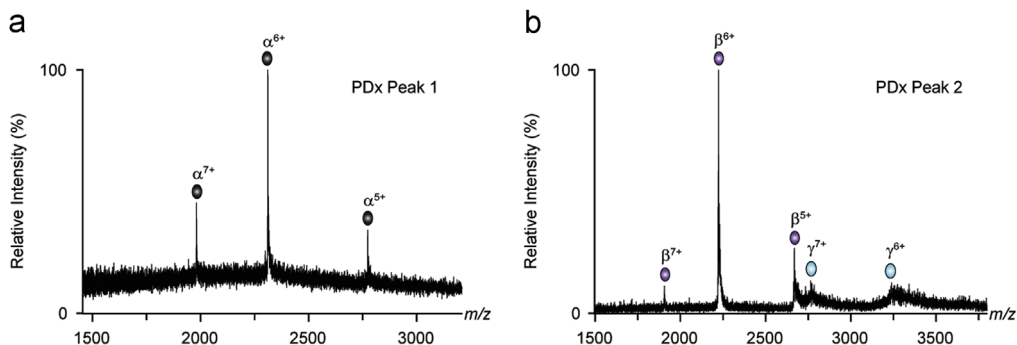


Fig. 2. Mass spectra of PDx subunits separated by HPLC [1]. Spectra exhibit multiple charge states. The spectrum from the first HPLC peak (Figure 5 in [1]) for PDx (a) shows a charge state series between m/z 1750 and 2800 while the second HPLC peak (Figure 5 in [1]), (b) shows two series of charge states between m/z 1750 and 3350. The subunit identity of each series is shown above the peaks (α , β , and γ).

Coomassie Blue for at least 2 h, followed by destaining until protein bands were visible. Gels were scanned using a GS-800 calibrated densitometer (Bio-Rad, California, U.S.A.).

2.2. High pressure liquid chromatography (HPLC)

To separate individual subunits, PDx and TPx were denatured in a solution of 6 M guanidinium hydrochloride (GuHCl) for 1 h then centrifuged for 10 min at 16,000g in a SIGMA 1-14 microfuge (SciQuip, Shrewsbury, UK). A 100 μ L aliquot of each solution was loaded onto a Jupiter 5 μ m C18 300 \AA (150 \times 4.6 mm) reverse-phase chromatographic column (Phenomenex, California, USA) equilibrated with Solution A (0.085% TFA in water (v/v)), using a Shimadzu VP Series HPLC System (Kyoto, Japan). Fractions were eluted (0.5 mL/min) using an acetonitrile gradient: 5–35% solution B over 20 min, 35–45% B over 40 min, 45–95% B over 20 min (Solution B: 0.085% TFA in acetonitrile (v/v)). HPLC fractions were lyophilized and stored at -20°C until required.

2.3. Mass spectrometry analysis of HPLC samples

All spectra were acquired using a Synapt (G1) HDMS time-of-flight mass spectrometer (Waters, Manchester, UK) and calibrated against a spectrum of CsI solution (10 mg/mL) acquired on the same day. Samples from HPLC separations were dissolved in 200 mM NH_4OAc and introduced into the vacuum region of the instrument by electrospraying 2 μ L of solution from a gold-coated borosilicate nano-ESI capillary (prepared in-house). Conditions for the acquisition of mass spectra were as follows: capillary voltage, 1.5 kV; sample cone, 140 V; extraction cone, 4 V; trap collision 12.5 V; transfer

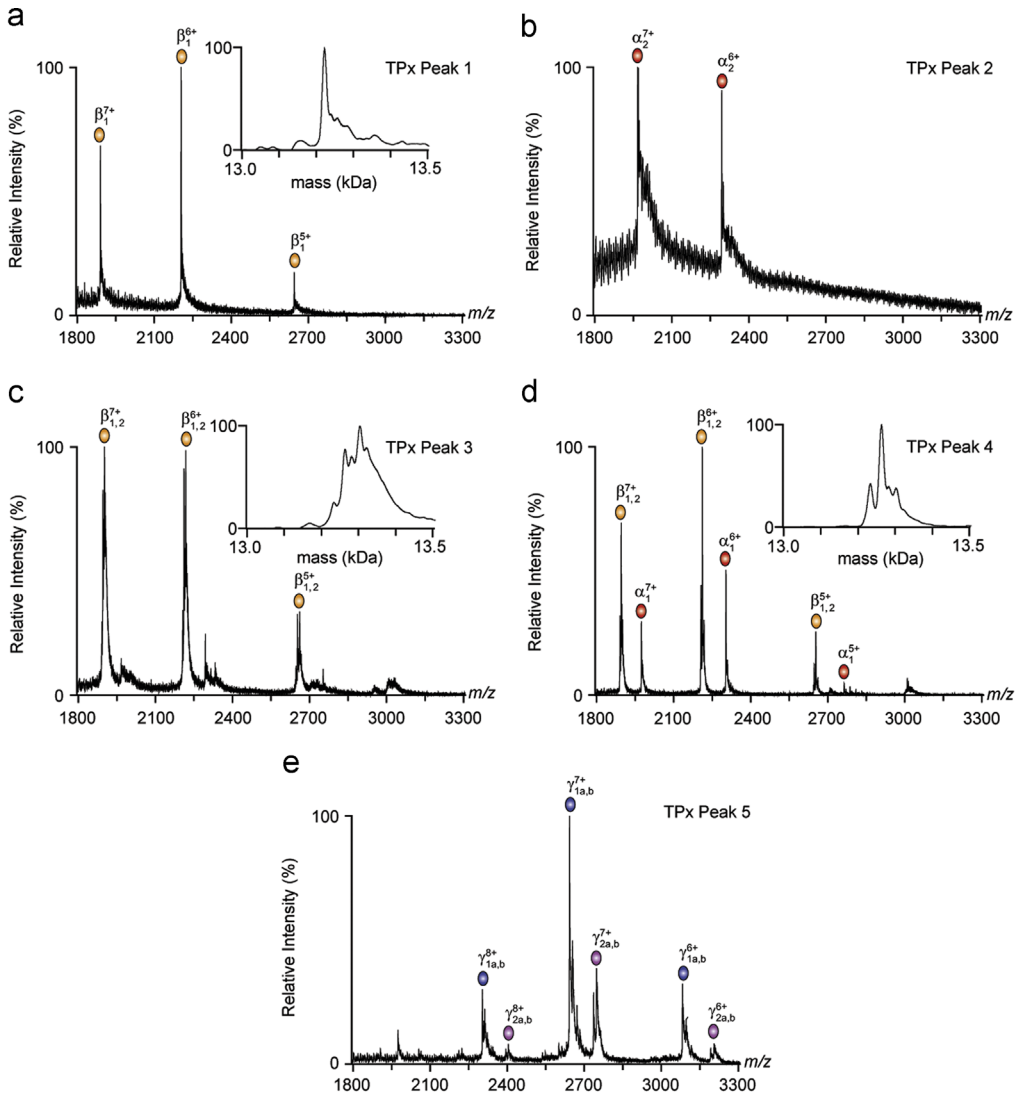


Fig. 3. Mass spectra of TPx subunits separated by HPLC [1]. For HPLC peaks one to four (Fig. 5 in [1]), charge state series were evident between 1800 and 2750 m/z , while peak 5 (Figure 5 in [1]) contained components with charge states arising within a higher m/z window. Each inset shows the mass distribution of β_{TPx} isoforms for relevant HPLC peaks. The subunit identified by each series is shown on the top of each spectra.

collision 25 V; collision cell gas pressure, 1.16×10^{-2} mbar; backing gas, 4.02×10^0 mbar. Mass spectra of the subunits of the toxins are shown in Figs. 2 and 3; the masses determined from the spectra are shown in Tables 1 and 2. These data were then compared to the existing sequence data for these toxins (Table 3).

Table 1
Nano-ESI-MS analysis of PDx components separated using HPLC. The number of each HPLC peak (Fig. 5 in [1] and Fig. 2 above) is shown in the left column of the table. The right column shows the observed mass of each protein detected in the nano-ESI-MS analyses, with the proposed identity in brackets.

Peak	Masses (Da) and Identity
1	13 862 (α)
2	13 318 (β)
	19 346 (γ)

Table 2
Nano-ESI-MS analysis of TPx components separated using HPLC. The number of each HPLC peak (Fig. 5 in [1] and Fig. 3 above) is shown in the left column of the table. The central column shows the observed mass of each protein detected in the nano-ESI-MS analyses, with the proposed identity in brackets. On the right, possible modifications to the disulfide linkages are proposed to account for differences between our findings and data in the published literature [4–7].

Peak	Masses (Da) and Identity	[S–S] _n
1	13 222 (β_1)	7
2	13 760 (α_2)	7
3, 4	13 264 (β_1)	6 + [Na ⁺] ₂
	13 303 (β_2)	5 + [H ⁺] ₄
	13 232 (β_1)	2 + [H ⁺] ₁₀
5	18 492 (γ_1a)	
	18 572 (γ_1b)	
	19 149 (γ_2a)	
	19 229 (γ_2b)	

Table 3
Previously published amino acid sequences of PDx and TPx subunits [4–8]. Table shows the identity of the subunits that were detected using mass spectrometry. The left most column shows the subunit, the columns to the right show the subunit sequence and mass (sequence summed and with cysteine bonds intact) respectively.

Subunit	Amino Acid Sequence	Mass (Da)
PDx β	NLLQFGFMIECAIRNRQPALDFMNYGCYCGTVGHGTPVDDLDRCCKTRNECYAEA EKHGCPYSLTTYRWQCGRVGLHCNSKTQCEVFVCACDLAAAKCLAQEDYNPAHFNINTKARCR[8]	Sequence: 13332 Cys bonds: 13318
TPx α	NLLQFGFMIRCANRRSRPVWHYMDYGCYCGKGGSGTPVDDLDRCQVHDECYGEAVRR FGCAPYWTLYSWKCYGKAPTCTNKTTRCQRFVCRCDAAAECFARSPYQNSNWNINTKARCR[6]	Sequence: 13829 Cys bonds: 13815
TPx β_1	NLVQFGKMIECAIRNRPALDFMNYGCYCGKGGSGTPVDDLDRCQVHDECYAEAEKHGCPYSLTTYTWECR QVGPCYNSKTQCEVFVCACDFAAAKCFQAQEDYNPAHSNINTGERCK[4,7]	Sequence: 13236 Cys bonds: 13222
TPx β_2	NLVQFGFMIECAIRNRPALDFMNYGCYCGTVGRGTPVDDLDRCQVHDECYATAEKHGCPYSLTTYQWEC RQVGNECNSKTQCEVFVCACDLAAAKCLAQEDYNPAHFNINTGERCK[4]	Sequence: 13313 Cys bonds: 13229
TPx γ	SEIQPSLDFEQFSNMIQCTIPCGESCLAYMDYGCYCGPGSGTPIDDLDRCKTHDECYAEAGKL SACKSVLSEPNNDTYSYECNEGQLTCNDNDNECKAFICNCDRTAVTCFAGAPYNDLNYNIGMIEHCK[5]	Sequence: 14602 Cys bonds: 14588

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Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dib.2016.09.005>.

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